Cell Stem Cell
Letters

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Response to Legraverend et al.

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I thank Drs. Legraverend, Escobar, and Jay for their interest in our recent manuscript, in particular our data showing asymmetric segregation of label-retaining DNA in dividing cells in the stem cell compartment of the intestine. I welcome the opportunity to respond to the specific questions raised in the letter about our study (Quyn et al., 2010) and about whether our data support Cairns' hypothesis or alternative interpretations are possible.

As Legraverend et al. (2010) discuss, asymmetric DNA segregation was previously observed in mouse gut epithelium by Potten et al. (2002) using a labeling protocol related to ours and 2D, sectioned tissue material. In our view, our data are entirely consistent with and build on the findings described in the previous analysis, and any numerical discrepancy is likely a result of the difference in protocols used. Importantly, we used 3D imaging of whole tissue, which permits examination of entire mitotic figures in the context of whole tissue from all angles, and thus excludes potential sectioning artifacts. We use this type of analysis to count the number of mitotic cells that unambiguously segregate their labeled DNA asymmetrically and also record differences in stem cell versus non-stem cell compartments. This type of quantitation was not performed previously.

Legraverend et al. also raise the possibility that the long-term label retention in the stem cell compartment observed in Quyn et al. (2010), by Potten et al. (2002), and by Falconer et al. (2010) in their recent related paper reflects the asymmetric segregation of a unique subset of chromosomes. However, the EdU label in our dividing cells is very clearly restricted to only the basal side of dividing cells and in these cases, all the DNA on the basal side is labeled with EdU as shown by perfect overlay with DAPI. These data seem inconsistent with the idea that only a subset of chromosomes is labeled. Please note that in symmetrically dividing, label-retaining cells we commonly observed patchy EdU distribution, suggesting the we can detect subsets of chromosomes with this method.

Lastly, Legraverend et al. raise questions about whether the asymmetry we observed was induced by the radiation used for eliminating stem cells and might reflect a cellular response to injury. In our opinion, this is unlikely for a number of reasons: (1) Tissue is analyzed 11 days after the radiation event (3 days of labeling plus 8 days of recovery). At this stage the tissue is completely normal in appearance and function. (2) Asymmetric segregation correlates perfectly with asymmetric alignment of mitotic spindles, which is detected in nonirradiated tissue. (3) Division in the non-stem cell compartment (above position +4) rarely showed asymmetric segregation, and (4) tissue from $Apc^{Min+/-}$ mice did not show asymmetric segregation, suggesting that it is not a general consequence of radiation treatment. Moreover, as Legraverend et al. discuss, Falconer et al. used an entirely different approach that did not involve injury and yet also observed nonsymmetric DNA segregation.

We completely agree that the underlying mechanism for asymmetric segregation/division, the biological relevance for cancer, and the relationship to stem cell maintenance are key questions to tackle in future research and that a combination of tools that take into consideration the issues raised by this discussion are required to address these issues.

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